Molecular Breeding Research with Sugar Beet

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Abstract

Breeding research in sugar beet (*Beta vulgaris* L.) is supported by a number of molecular tools. This paper gives a short overview of the most important tools and presents some of the molecular breeding research currently conducted at the University of Kiel including the establishment of a sugar beet TILLING platform, the cloning of beet cyst nematode resistance from *B. procumbens* and breeding of sugar beet as a winter crop.

Keywords:

sugar beet, *Beta vulgaris*, molecular breeding

Several genetic linkage maps of the 758 Mb sugar beet genome have been developed in the past years including a more recent map covering 664 cM and comprising 315 expressed sequence tag (EST) markers (SCHNEIDER et al. 2007). These maps have been extensively used for mapping of traits with agronomic importance (WEBER et al. 2000, SCHAFER-PREGL et al. 1999, GRIMMER et al. 2008, LEIN et al. 2007, JANSSEN et al. 2003, NILSSON et al. 1999, GIDNER et al. 2005, WEBER et al. 1999, SETIAWAN et al. 2000, SCHNEIDER et al. 2002, TAGUCHI et al. 2008, LEIN et al. 2008). Further resources are large insert libraries (HOHMANN et al. 2003, LANGE et al. 2008, SCHULTE et al. 2006) and about 26,000 publicly available ESTs of which more than 13,000 represent distinct genes (DFCI Beet Gene Index, 2008). Physical mapping and sequencing of the whole sugar beet genome is in progress (LANGE et al. 2008) and supported by high resolution FISH (fluorescent *in situ* hybridisation) resolving down to 1 kb (D. Dechyeva, personal communication). The complete *B. vulgaris* DNA sequence is expected to be available by 2011 and will provide a valuable tool for sugar beet genomics research. Genetic transformation of sugar beet has been established for many years (GUREL et al. 2008) although complete regeneration of transformants still remains challenging.

As an alternative to genetic transformation, EMS mutagenesis has been recently established at the University of Kiel (HOHMANN et al. 2005) in order to study gene function and to obtain *de novo* genetic variation in sugar beet germplasm. A TILLING platform (COMAI and HENIKOFF 2006) for sugar beet has been established allowing high throughput identification of mutations in candidate genes and their subsequent phenotypic characterization. A sugar beet mutant population is currently being screened for mutants with functional alteration in putative flowering genes and first mutants have been identified. This mutant population was generated from an annual sugar beet inbred line and comprises about 1,100 M2 families. A second mutant population generated from a biennial DH line is currently in work.

Cloning of resistance to beet cyst nematode (BCN, *Heterodera schachtii* Schmidt) has been an objective at the University of Kiel for more than 15 years leading to the cloning of the resistance gene $Hs1^{pro-1}$ (CAI et al. 1997). BCN is the most severe pest in sugar beet and the only sources of resistance are the wild species *B. procumbens* and the related species *B. webbiana* and *B. patellaris*. Two resistant sugar beet lines A906001 and TR363 carrying translocations from *B. procumbens* at the end of chromosome 9 are used for cloning a 2nd resistance gene $Hs1^{-1}$ which is tightly linked to the previously cloned resistance gene $Hs1^{pro-1}$. The size of the translocation from the line A906001 was estimated to be 1500 kb. A physical map of this translocation was established encompassing 18 BAC clones with a total length of their inserts of 1,415 kb. The BAC contigs are separated by 4 small gaps. Since this translocation is too big to find an individual gene we have created a 400 Gy gamma mutated population. 2670 seeds were irradiated and, after screening of 578 M1 offspring with three molecular markers evenly spread around the translocation, two mutants were found which had lost most of the translocation including the $Hs1^{-1}$ gene. By comparative mutant analysis the region housing the $Hs1^{-1}$ gene could be narrowed down to ca. 300 kb. The $Hs1^{-1}$ gene is presently being identified from the sequences of 3 BACs covering the critical region of the translocation. Complementation studies on sugar beet hairy roots will be conducted to determine the putative function of the candidate genes present in this region.

A promising strategy to increase the yield potential of sugar beet is to grow it as winter crop thus extending its vegetation period. However, this has not been achieved as sugar beet starts bolting after prolonged exposure to cold during winter (vernalization). The tendency for early bolting (without a requirement for vernalization) is under the control of a single dominant gene termed *B* which is currently being cloned from its position on chromosome 2 (HOHMANN et al. 2003, GAAFAR et al. 2005, MÜLLER and JUNG, unpublished data). As a result of strong selection against early bolting, commercial sugar beet cultivars do not contain a functional *B* allele and behave as biennials. In the absence of *B*, induction and timing of flowering depends on vernalization

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and requires appropriate photoperiodic and developmental conditions. The recent identification and functional analysis of sugar beet genes with homology to *FLC* and *CO*, two key floral transition genes in Arabidopsis, provide first evidence for conservation of the genetic basis of flowering time control in sugar beet (CHIA et al. 2008, REEVES et al. 2007). These genes and others that have been identified at the University of Kiel are prime candidates for targeted genetic approaches to suppress, or induce, flowering under controlled conditions.

In addition to controlled bolting behaviour, winter sugar beets also require sufficient winter hardiness to be grown as a winter crop and little research has been aimed at this until now. Winter hardiness in sugar beet is expected to be a complex trait comprising tolerance to frost and other abiotic stresses as well as resistance to biotic stresses exposed to with or without snow covering. Further, frost tolerance as the most obvious factor can be achieved by physiological plant characteristics such as osmotic potential and by morphological characteristics such as escape mechanisms conditioned by plant architecture. Currently, about 400 biennial *B. vulgaris* accessions sown in August are grown in a replicated overwintering field trial at two locations in Belarus and two locations in Germany. The accessions comprise the cultivated forms sugar beet, leaf beet (Swiss chard), red table beet and fodder beet as well as the wild beet *B. vulgaris maritima*. The objectives of this experiment is (i) to investigate the genetic variation of winter hardiness in the *B. vulgaris* genepool, (ii) to record plant architecture traits potentially affecting winter hardiness and (iii) to identify sources of winter hardiness that can be exploited for the development of winter sugar beets.

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